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TITLE: Dopamine D3 Agonists: Developing Treatments for Sexual Dysfunction in Chronic Spinal Cord-Injured Male Rats

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

Spinal cord injury (SCI) and resulting dysfunction is a major health concern for military men and has immense impact on quality of life for the patients and their families. Genitourinary dysfunction is extremely common among men with SCI. This dysfunction greatly hinders quality of life and emotional well-being and is thus of great concern to military men. Surveys of men with SCI have demonstrated that regaining sexual function is an important goal, even surpassing that for recovery of walking among paraplegic men. This is of particular relevance to the military as combat-related spinal trauma is the highest in American military history and the average age of spinal casualties is 26-27 years, hence issues impacting quality of life are of great importance. Thus, the development of novel, innovative interventions is of significant relevance for patients in both the military and civilian populations. Despite the identified need and desire to improve sexual function among SCI men, we have a poor understanding of the mechanisms by which chronic injury so radically influences the spinal ejaculation generator. The current proposal aims to fill that gap: studies detailed in this proposal will test the functional benefits of a novel treatment approach for ejaculatory dysfunction. Secondly, studies are proposed to move towards an improved understanding of the effects of chronic spinal injury on the overlapping neural circuits that regulate the ejaculation reflex. Together, these studies will be beneficial in developing treatments to restore sexual function and fertility to SCI men and importantly improve their overall emotional-well-being and quality of life. Since military men suffer SCI at a relatively young age, potential for treatments of these secondary dysfunctions and thus improving quality of life is highly relevant and impactful.

15. SUBJECT TERMS
None listed.

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1. Introduction

Spinal cord injury (SCI) is a devastating clinical injury with extreme cost and burden on patients and their families. Following initial stabilization after spinal cord injury, quality of life issues become increasingly important to patients. In particular, genitourinary dysfunctions are extremely common among SCI patients, including sexual dysfunction. Surveys of men with SCI have demonstrated that regaining sexual function is an important goal, even surpassing that for recovery of walking among paraplegic men. Unfortunately, treatment strategies to overcome genitourinary dysfunctions in SCI men are limited and invasive, and development of non-invasive treatments has been impaired due to poor understanding of the mechanisms by which chronic injury so radically influences the spinal reflex pathways. Moreover, ejaculatory dysfunction is of particular relevance to military patients, as combat-related spinal trauma is the highest in American military history and military men suffer SCI at a relatively young age. Hence, potential for treatments of these secondary dysfunctions and thus improving quality of life is highly relevant and impactful. Of particular relevance to the patient population and to this proposal is that preclinical and clinical studies that determine benefits of a potential treatment for sexual function simultaneously are sparse. The current proposal aims to fill that gap: studies detailed in this proposal will test the functional benefits of a novel treatment approach for ejaculatory dysfunction, using an established preclinical model and a pharmacological treatment that is already well-established for other clinical use. Secondly, studies are proposed to move towards an improved understanding of the impacts of chronic spinal injury on the neural circuits that regulate the ejaculation reflex. Such studies are needed to further advance the proposed treatment options in preclinical models towards clinical treatments.

2. Keywords

Spinal cord injury, sexual dysfunction, anejaculation, dopamine, contusion injury

3. Accomplishments

Major Goals:
Major Task 1: Study Regulatory Set-up
Major Task 2 (Specific Aim 1A): To test the hypothesis that acute treatment with D3 agonist pramipexole restores ejaculatory function following SCI.
Major Task 3 (Specific Aim 1b): To test the hypothesis that chronic treatment with D3 agonist pramipexole restores ejaculatory function following SCI.
Major Task 4 (Specific Aim 2): To examine localization of D3 receptors in and dopaminergic innervation of neurons controlling ejaculatory reflexes, and effects of SCI on dopaminergic transmission.

Accomplishments under these goals:
Major Task 1: Study Regulatory Set-up:
Subtask 1.1: Submit documents to the Kent State University IACUC and obtain approval.
Progress: This was not accomplished during the reporting period (but has been submitted since).

Subtask 1.2: Submit IRB approval documents and animal protocol to DoD's ACURO and make revisions as necessary.
Progress: This was not accomplished during the reporting period (current pending KSU IACUC approval).
Subtask 1.3: Hire and train study personnel on RNAscope, immunofluorescent staining techniques, confocal microscopy, fluorescent microscopy, animal surgeries, pharmacological and physiological procedures
Progress: Research technician was assigned to this project in June 2022 and Graduate Research Assistant was appointed to these projects in August 2022. Both new personnel has been trained on RNAscope, immunofluorescent staining techniques, confocal microscopy, fluorescent microscopy. Graduate student was trained on animal surgeries, pharmacological and physiological procedures.

Major Task 2 (Specific Aim 1A): To test the hypothesis that acute treatment with D3 agonist pramipexole restores ejaculatory function following SCI.
Progress: These studies have not yet started and are planned for the second budget year.

Major Task 3 (Specific Aim 1b): To test the hypothesis that chronic treatment with D3 agonist pramipexole restores ejaculatory function following SCI.
Progress: These studies have not yet started and are planned for the second and third budget year.

Major Task 4 (Specific Aim 2): *To examine localization of D3 receptors in and dopaminergic innervation of neurons controlling ejaculatory reflexes, and effects of SCI on dopaminergic transmission.*
Subtask 4.1: Complete sham and SCI surgery at T5-6 levels and postoperative care. Determine locomotor activity weekly. At 2, 6, or 12 weeks after sham or SCI surgeries, perfuse animals with 4% paraformaldehyde and collect spinal cords, brains, and sperm. 6 groups of rats will be included, each containing N=8 rats; hence N=48 animals are included in Aim 2.
Progress: This study hasn't started and animal protocol is pending.
Subtasks 4.2, 4.3, 4.4 will start once 4.1 has been completed.

Subtask 4.5: Process spinal cord sections will be processed for FISH using RNAscope probes for rat *D3*, *galanin* and *ChAT*.
Subtask 4.6. Capture confocal and fluorescent microscope images and contact image analyses.
Progress: Spinal cord sections that were stored from a prior experiment including Sham and Spinal cord injured (SCI) rats, with identical treatment to what is proposed in this award, were processed using RNAscope for dopamine 3, dopamine 2, and dopamine 1 receptors together with galanin (marker for spinal ejaculation generator cells, which are referred to as lumbar spinothalamic or LSt cells). This study was designed as a pilot study and results are thus preliminary. Confocal analysis showed that approximately 45-70% of LSt express D3 receptors, with only 2-5% expressing D2 and 2-7% expressing D1 receptors. Hence, D3 receptors are the primary dopamine receptor expressed on LSt cells. Moreover, there was a significant trend towards decreased expression of D3 receptors in LSt cells of SCI males. These results are promising. However, since the study was a pilot study and with limited numbers of animals and cells included in analysis, this experiment needs to be replicated in the second budget year.

Subtask 4.3: Process spinal cord sections for immunofluorescence.
Progress: Antibodies have been purchased and protocols have been developed for immunofluprescent detection of TH in combination with galanin (marker for LSt cells) or ChAT and together with synaptophysin using Dylight 488, 550 and 630-conjugated secondary antibodies.

Opportunities for training and professional development
Training and development was offered to newly hired research technician and to PhD student.

Dissemination of results:
Nothing to report

Plans during next reporting period:
Subtask 1.1: Submit documents to the Kent State University IACUC and obtain approval.
Subtask 1.2: Submit animal protocol to DoD's ACURO and make revisions as necessary
Subtask 2.1: Complete sham and SCI surgeries, measure locomotor activity, and administer the D3 receptor agonist pramipexole at one of three dosages (0.1, 0.3 or 1 mg/kg) or saline vehicle at 2 weeks post-surgery. Sexual reflexes will be examined. This will include 8 groups (12 rats/group) of male Sprague Dawley rats for a total N = 96

Subtask 4.1: Complete sham and SCI surgery at T5-6 levels and postoperative care. Determine locomotor activity weekly. At 2, 6, or 12 weeks after sham or SCI surgeries, perfuse animals with 4% paraformaldehyde and collect spinal cords, brains, and sperm. 6 groups of rats will be included, each containing N=8 rats; hence N=48 animals are included in Aim 2.
Subtask 4.2: Section Spinal cords coronally at 14 μm using cryostat and collected in parralel series on microscope slides.
Subtask 4.3: Process spinal cord sections for immunofluorescence for TH, galanin and synaptophysin.
Subtask 4.4. Capture confocal and fluorescent microscope images and contact image analyses.

4. Impact

Nothing to report

5. Changes/Problems

Actual or anticipated problems or delays and actions or plans to resolve them:
There were some delays in hiring personnel, largely due to effects of the pandemic on workforce. This has been resolved and two full time personnel are now conducting these studies.

6. Products

Nothing to report

7. Participants & Other Collaborating Organizations

Name:	*Lique M Coolen*
Project Role:	*Project Director*
Researcher Identifier (e.g. ORCID ID):	*ORCID ID 0000-0003-2920-1116*
Nearest person month worked:	*1.2*
Contribution to Project:	*Dr. Coolen oversees all aspects of the projects and has supervised and trained the Research Technician and graduate student.*
Funding Support:	*N/A*

Name:	*Rachel Rice*
Project Role:	*Research Technician*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month	*2*

worked:	
Contribution to Project:	*Ms. Rice conducts RNAscope, confocal microscopy and image analysis*
Funding Support:	*N/A*

Name:	*Thywill Ettey*
Project Role:	*PhD Student- Graduate Assistant-Research Assistant*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*1*
Contribution to Project:	*Ms. Ettey is involved in all aspects of these studies*
Funding Support:	*N/A*

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
No changes:

What other organizations were involved as partners?
Nothing to Report

8. Special Reporting Requirements

9. Appendices